

CLAIMS

1. A method for producing an *in vitro* peptide expression library comprising a plurality of peptides, wherein each peptide is linked to the DNA construct encoding the peptide, comprising the steps of:
 - (a) providing a DNA construct comprising:
 - (i) a DNA target sequence;
 - (ii) DNA encoding a library member peptide; and
 - (iii) DNA encoding a peptide capable of non-covalently binding directly or indirectly to said DNA target sequence of (i);wherein said DNA construct and encoded protein are selected to have cis-activity
 - (b) expressing a plurality of DNA constructs according to (a) wherein said DNA constructs encode a plurality of library member peptides such that each expressed peptide is non-covalently linked to the DNA from which it was produced.
2. A method according to claim 1 wherein said DNA construct further comprises:
 - (iv) a DNA element that directs cis-activity.
3. A method according to claim 2 wherein said DNA construct of (a) further comprises
 - (v) DNA encoding a fragment comprising at least the C-terminal 20 amino acids of a repA protein wherein said fragment is capable of interacting with said DNA element of (iv);wherein said DNA element of (iv) is located 3' to said DNA of (ii), (iii) and (v).
4. A method according to any one of the preceding claims wherein the peptide encoded by said DNA of (iii) is capable of recognising and directly binding said DNA target sequence of (i).
5. A method according to claim 4 wherein the peptide encoded by said

DNA of (iii) is a repA protein and wherein said DNA target sequence of (i) is ori.

6. A method according to claim 4 or 5 wherein said DNA of (ii) is linked to said DNA of (i) and (iii) by restriction enzyme digestion and ligation.

7. A method according to any one of claims 3 to 6 wherein said repA is selected from repA of the IncI complex plasmids and repA of the IncF, IncB, IncK, IncZ and IncL/M plasmids.

8. A method according to claim 5 wherein said DNA construct comprises the sequence encoding repA, the cis DNA element and the ori DNA of the IncFII plasmid R1.

9. A method according to any one of the preceding claims wherein said repA protein has the sequence given in SEQ ID NO: 16 and wherein said cis DNA element has the sequence given in SEQ ID NO: 17.

10. A method according to any one of the preceding claims wherein DNA not bound by the peptide encoded by said DNA of (iii) is bound by non-specific DNA binding protein.

11. A method according to claim 4 wherein the peptide encoded by said DNA of (iii) is an oestrogen receptor DNA binding domain and wherein said DNA target sequence of (i) is an oestrogen receptor target sequence.

12. A method according to claim 9 wherein said DNA binding domain comprises amino acids 176 to 282 of the oestrogen receptor DNA binding fragment and wherein said DNA target sequence comprises the oestrogen receptor target sequence given in SEQ ID NO: 14.

13. A method according to claim 1, 2 or 3 wherein the peptide encoded by said DNA of (iii) indirectly binds said DNA target sequence of (i) via a bifunctional agent, one part of which binds said DNA target sequence of (i) and a second part of which binds the peptide encoded by said DNA of (iii).

14. A method according to claim 13 wherein said DNA target sequence comprises a DNA tag capable of being bound by said bifunctional agent, said tag being optionally selected from biotin and fluorescein.

15. A method according to claim 13 or 14 wherein the binding activities of said bifunctional agent are conferred by means of two antibodies or fragments thereof.

16. A method according to claim 15 wherein one or both of said binding activities are conferred by means of an Fab fragment.

17. A method according to any one of claims 13 to 16 wherein said bifunctional agent is provided prior to step (b).

18. A method according to claim 13 wherein said bifunctional agent is bound to said DNA target sequence of (i) and is capable of binding to the peptide encoded by said DNA of (iii).

19. A method according to claim 18 wherein said bifunctional agent is a polymer.

20. A method according to any one of the preceding claims wherein said DNA is under the control of suitable promoter and translation sequences to allow for *in vitro* transcription and translation.

21. A method according to any one of the preceding claims wherein said library member peptide is an antibody or fragment thereof.

22. A method according to any one of the preceding claims wherein said library comprises at least 10^4 molecules.

23. A method according to any one of the preceding claims wherein said expression is carried out in the presence of a compound that prevents nuclease activity, or reduces non-specific DNA-protein or protein-protein interactions.

24. A method according to any one of the preceding claims wherein said expression is carried out in a coupled bacterial transcription/translation environment.

25. A method according to claim 24 wherein said coupled bacterial transcription/translation environment is the S30 extract system.

26. A method for producing an *in vitro* peptide expression library comprising a plurality of peptides, wherein each peptide is linked to the DNA construct encoding the peptide, comprising the steps of:

- (a) providing a DNA construct comprising:
 - (i) DNA encoding a library member peptide; and
 - (ii) DNA encoding a peptide capable of binding to a bifunctional agent;wherein said DNA construct and encoded protein are selected to have cis-activity;
- (b) binding a bifunctional agent or a DNA tag capable of binding a bifunctional agent to said DNA construct of (a), wherein said bifunctional agent is capable of binding to the peptide encoded by said DNA of (ii); and
- (c) expressing a plurality of DNA constructs according to (b), wherein said DNA constructs encode a plurality of library member peptides

such that each expressed peptide is linked via said bifunctional agent to the DNA from which it was produced.

27. A method of identifying and/or purifying a peptide exhibiting desired properties from an *in vitro* peptide expression library produced according to the method of any one of the preceding claims, comprising at least the steps of (a) screening said library and (b) selecting and isolating the relevant library member.

28. A method of identifying a specific ligand binding peptide, said method comprising at least the steps of (a) screening an *in vitro* peptide expression library produced according to the method of any one of claims 1 to 26 with ligand molecules which are optionally bound to a solid support; (b) selecting and isolating a library member binding to said ligand molecule; and (c) isolating the peptide which binds specifically to said ligand molecule.

29. A method according to claim 27 or 28 wherein said library member peptides are antibodies or fragments thereof.

30. A method of identifying and/or purifying a peptide having the ability to bind a specific DNA target sequence comprising at least the steps of

- (a) providing an *in vitro* expression library according to any one of claims 1 to 26 wherein the peptide encoded by the DNA of (iii) is a library member peptide having DNA binding activity and wherein said DNA target sequence of (i) is the target sequence of interest;
- (b) selecting and isolating a library member in which the encoded protein binds to said target sequence; and
- (c) isolating the peptide which binds to said target sequence.

31. A method according to claim 30 wherein said library member peptides are zinc finger proteins, helix-loop-helix proteins or helix-turn-helix proteins.

32. A method according to any one of claims 27 to 31 wherein said screening and/or selecting step is carried out in the presence of a compound that prevents nuclease activity or reduces non-specific DNA-protein or protein-protein interactions.
33. A method according to claim 32 wherein said compound is heparin.
34. A method according to any one of claims 27 to 33 wherein additionally the DNA expressing said isolated peptide is isolated.
35. A method according to claim 34 further comprising cloning said DNA into an expression vector.
36. A method according to claim 35 further comprising introducing said expression vector into a cell *in vitro*.
37. A method according to claim 35 or 36 further comprising expressing the peptide encoded by said DNA.
38. An *in vitro* peptide expression library produced according to the method of any one of claims 1 to 26.
39. A DNA construct as described in any one of claims 1 to 26.